Supplemental Figure 1: Endothelial progenitor cells characterisation in vitro.

Early (□, 7 days) and late (■, 14 days) endothelial progenitor cells from MA- and MA+ diabetic patients used in the tube formation assay were characterised by immunofluorescence as described (method section). Human umbilical vascular endothelial cells and macrophage line U937 were utilised in parallel as positive control for endothelial and monocytic markers respectively, negative control were included by omitting the first antiserum. Non-specific Fc receptors were blocked using blocking buffer (CAS block, Zymed laboratories-Invitrogen, Paisley, UK). Primary antisera (Santa Cruz Biotechnology, SantaCruz, CA, USA) and secondary AlexaFluor488 or Texas Red antisera were used at 1/50 dilution (ChemMate[™] Antibody Diluent, Dako, Ely, UK). Data are expressed as mean±SD % cells stained with specific antigen. Cells were stained in duplicate, four different field (~20-30 cells) were assessed by a blinded investigator and the mean used in the calculations (n=7 for MA-, n=9 for MA+).

No differences were observed in cell phenotype between the MA- and MA+ group. At 7 days culture, cells expressed mainly VEGFR2, eNOS and the monocytic specific markers CD45 and CD14. At 14 days culture, cells expressed more endothelial specific markers (VEGFR2, CD144, Von Willebrand Factor-VWF, CD31, and eNOS) while we observed a reduction in cells positive for CD14. Cells (~80%) in both groups expressed CD45 at 14 days.

Stem cell markers CD34 and CD133 were positive in a small proportion of cells (~2-5%).



